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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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ANALYSIS OF STROMAL CELL CULTURE PROLIFERATION BIOMARKER USING MEDICAL ADHESIVES

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Abstract.

Introduction: Medical adhesives are widely used in surgical dentistry, including for covering donor sites after free gingival graft harvesting. However, their cytotoxic properties and effects on tissue proliferative activity remain a topic of scientific debate.

Objective: To evaluate the effects of various adhesive compositions on the proliferative activity of human oral mucosa stromal cells using Ki-67 staining, and to analyze apoptotic and necrotic changes.

Materials and Methods: Three types of medical adhesives were investigated: feracryl-based (Hemocompact), domestic cyanoacrylate (Sulfacrylate), and imported cyanoacrylate (Histoacryl). Adhesives were applied to sterile polycarboxylate plates seeded with human stromal cells. After 24–48 hours of incubation, morphological evaluation, apoptosis and necrosis analysis using Annexin V-FITC/PI, and Ki-67 expression analysis by flow cytometry were per-formed. Statistical analysis included ANOVA and Tukey's post hoc test ($p<0.05$).

Results: Feracryl samples showed the highest cytotoxicity: elevated necrosis (16.65%) and reduced Ki-67 expression ($p<0.001$). Cyanoacrylate-based adhesives showed better biocompatibility: Histoacryl demonstrated the lowest necrosis rate and best cell morphology preservation. Both null hypotheses were rejected.

Conclusion: Cyanoacrylate-based adhesives, especially imported ones, are safer for use in areas with high regenerative activity. Feracryl-based adhesives are limited due to their significant cytotoxicity. The findings support the use of specific adhesives as wound coverings for donor sites after soft tissue graft harvesting.

Key words. Ki-67, medical adhesives, stromal cells, apoptosis, proliferation, cytotoxicity, flow cytometry.

Introduction.

In modern dental surgery, medical adhesives have gained widespread popularity due to their ease of use, hemostatic properties, and ability to accelerate tissue healing. However, despite the wide variety of available adhesive compositions and their extensive clinical application, the degree of their cytotoxicity remains a subject of scientific debate. The literature describes both positive and negative effects of cyanoacrylate-based and other adhesive components, highlight-ing the need for further in vitro investigations to assess their biocompatibility and safety [1,2].

One of the common clinical scenarios in which medical adhesives are applied is their use as a protective covering for the palatal donor site after harvesting a free gingival graft. This procedure requires a high degree of regenerative capacity of the oral mucosa; therefore, evaluating the adhesive's effect on tissue cells becomes critically important. Impairment of cell proliferation, induction of apoptosis, or the development of necrosis can significantly affect the timing and quality of wound healing and, in some cases, lead to postoperative complications [3,4].

To assess the safety and biocompatibility of adhesive materials, it is essential to employ mod-ern molecular and biological approaches, one of which involves analyzing cellular proliferative activity. The Ki-67 marker is a nuclear antigen expressed during the active phases of the cell cycle and is widely recognized as a standard indicator of cell proliferation. Simultaneously, the evaluation of apoptotic and necrotic changes provides insight into the potential toxicity of materials and the mechanisms of cell death [5,6].

In experimental settings, the use of primary cultures of human oral mucosal stromal cells is of particular value, as these are the first cells to come into contact with adhesive materials in actual clinical scenarios. The application of flow cytometry, which enables quantitative assessment of Ki-67 expression, apoptosis, and necrosis levels, ensures a high degree of reliability in the obtained data [7,8].

Objective: To determine the proliferative activity of a stromal cell culture derived from a biopsy of the human oral floor mucosa using the Ki-67 marker. Additionally, to analyze the dynamics of changes in the percentage of necrosis and different types of apoptosis depending on the tested samples.

The following null hypotheses were formulated for this study:

1. **H_{01}:** The use of different types of medical adhesives does not have a statistically significant effect on the proliferative activity of oral mucosal stromal cells, as measured by the Ki-67 marker.
2. **H_{02}:** Different medical adhesives do not cause statistically significant differences in the levels of apoptosis and necrosis in the culture of oral mucosal stromal cells.

Materials and Methods.

Characteristics of the Investigated Materials:

The study included three types of medical adhesives that differ in composition and manufacturer. Two of them were cyanoacrylate-based (the domestically produced Sulfacrylate and the imported Histoacryl), and one was based on an aqueous solution of feracrylate (the domestically produced Hemocompat). These adhesives are widely used in surgery; however, their biocompatibility in the context of oral soft tissue regeneration remains a topic of scientific interest. The comparison was conducted considering their chemical composition, form of release, and prevalence in clinical practice. A detailed description of the adhesive compositions

Table 1. *Characteristics of the Investigated Adhesive Compositions.*

Adhesive	Manufacturer	Composition	Group
Hemocompat	LLC "MTPO Inter-Vita", Moscow, Russia	- Aqueous solution of feracrylate 1% - Sodium alginate - Acetic acid (chemically pure) for pharmaceutical use, purity ≥99.85% (GOST 61-75, GOST 2654-86) - Distilled water for pharmaceutical use	Feracrylate
Sulfacrylate	LLC "NTO MedLin", Novosibirsk, Russia	- Ethyl ester of α-cyanoacrylic acid (responsible for polymerization upon contact with aqueous media) - Butyl ester of acrylic acid (adds plasticity) - Methacrylate-3-oxysulphalan (provides antimicrobial activity and promotes rapid wound healing)	Cyanoacrylate
Histoacryl	B. Braun, Germany	Monomer of n-butyl-2-cyanoacrylate	Cyanoacrylate

is presented in Table 1. Each experimental condition, including the control group (cells cultured without adhesive materials), was represented by three independent biological replicates to ensure data reliability.

Sample Preparation.

Each sample was prepared using a polycarboxylate plate with a diameter of 2.5 mm and a thickness of 1 mm. A total of nine samples were prepared—three for each of the tested adhesive compositions. The application of the adhesive was performed under sterile conditions, with the adhesive carefully distributed in an even layer across the surface of the plates. The samples were then left to dry completely at room temperature in a sterile environment. Prior to biological testing, all samples were subjected to UV irradiation for sterilization. No contamination of the samples with bacteria, fungi, or other microorganisms was observed.

Cell Model.

To assess the biocompatibility of the samples, a primary culture of stromal cells from the hu-man oral floor mucosa was used. Tissue collection was conducted in accordance with ethical standards and after obtaining written informed consent from the patient. Biopsy specimens were processed using a standard enzymatic digestion method, followed by cultivation in DMEM/F12 medium (Paneco, Russia) supplemented with 10% fetal calf serum (Capricorn Scientific, Germany) and 1% antibiotic (penicillin-streptomycin, Paneco, Russia). The cells were incubated at 37°C in a 5% CO_2 atmosphere until the required confluency was reached.

For the experiment, the cells were detached from the substrate using a trypsin-EDTA solution, and their count and viability were assessed using the Luna-fluo automated cell counter (Logos Biosystems, USA). The cells were then seeded into the wells of a 48-well plate, where the adhesive samples had already been placed. A total of 30,000 viable cells in 500 µL of medium were added to each well. After 48 hours of incubation, further evaluation was performed.

Cell Viability Assessment (Annexin V-FITC/PI).

To assess the levels of apoptosis and necrosis, a standard annexin-propidium iodide assay was used (Annexin V-FITC/PI Apoptosis Detection Kit, Vazyme, China). After 24 hours of incubation, the cells were detached from the substrate, washed twice in phosphate-buffered saline by centrifugation (300 g, 5 minutes), and resuspended in binding buffer at a concentration of 1×10^6 cells/mL. Staining was performed strictly according to the manufacturer's instructions. The analysis was carried out by flow cytometry using a MACSQuant 10 cytometer (Miltenyi Bio-tec, Germany). Four cell populations were identified: viable cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), late apoptotic cells (Annexin V^+/PI^+), and necrotic cells (Annexin V^-/PI^+).

Cell Proliferation Assessment (Ki-67).

To analyze proliferative activity, the Inside Stain Kit (Miltenyi Biotec, Germany) was used in combination with monoclonal antibodies against the nuclear proliferation marker Ki-67, conjugated with the fluorochrome FITC. After incubation, the cells were collected, fixed, permeabilized, and incubated with the antibodies for 30–60 minutes in the dark at room temperature. Following washing, the analysis was performed on the same MACSQuant 10 flow cytometer. Gating was carried out based on a comparative analysis of stained versus unstained samples.

Microscopy.

Additionally, morphological evaluation of the cells was performed using the ZOE inverted im-ager (Bio-Rad, USA). Photographic documentation of the cellular state allowed visualization of the degree of cell attachment, the presence of spheroid structures, and overall cell morphology in each experimental group.

Statistical Data Analysis.

All experimental data were analyzed using GraphPad Prism 9.0 software (GraphPad Software, USA). The Shapiro–Wilk test was used to assess the normality of data distribution. For normal-ly distributed data, intergroup comparisons were performed using one-way ANOVA followed by Tukey's post hoc test for pairwise comparisons. In cases where the data deviated from normality, non-parametric tests were applied (e.g., Kruskal–Wallis test).

The results are presented as mean ± standard deviation (M ± SD). A p-value of less than 0.05 was considered statistically significant. Graphical representations of the data included bar charts with indications of statistical significance between groups. Each measurement was per-formed in at least three biological replicates.

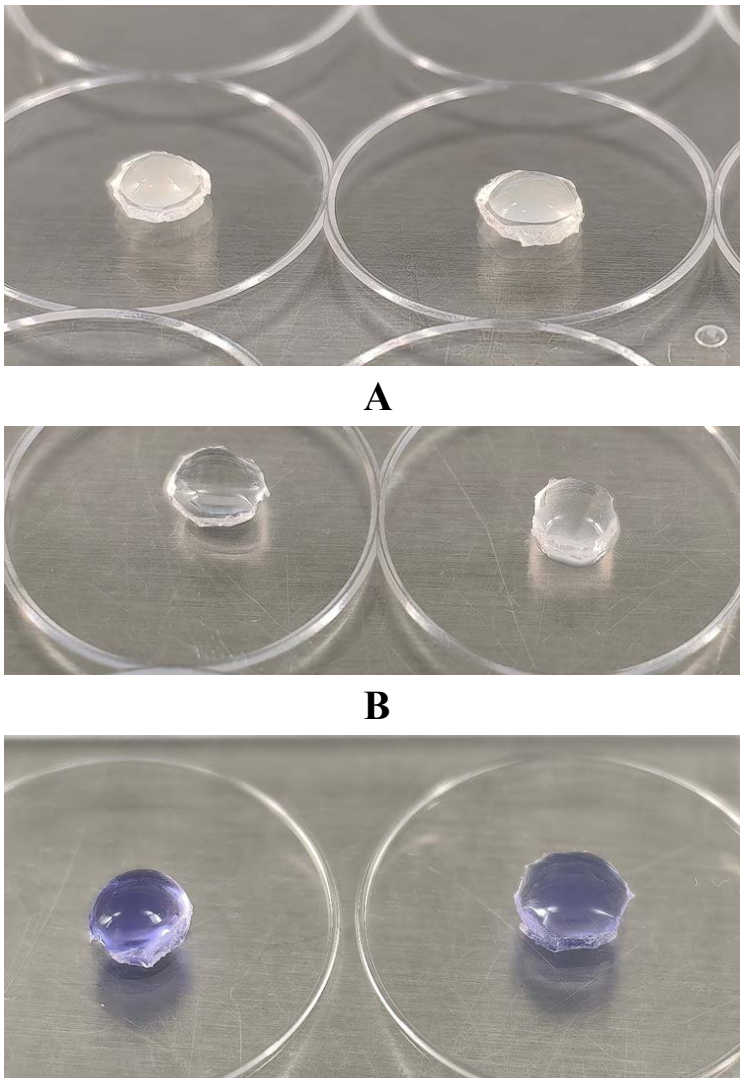


Figure 1. *Distribution of samples on polycarboxylate plates. A – Hemocompat, B – Sulfacrylate, C – Histoacryl.*

Figure 2. *Distribution of samples in a 12-well plate. A monolayer of cells without adhesive samples was used as the control.*

Results.

Morphological Characterization of Cells After Incubation with Adhesive Samples:

Microscopic examination using the ZOE Bio-Rad inverted imager revealed pronounced differences in cell morphology and adhesion between the experimental groups. During incubation with samples from Group 1 (Hemocompat), the cells exhibited a loss of adhesion capability and formed spheroid aggregates, indicating unfavorable environmental conditions and potential toxic effects of the adhesive. In Group 2 (Sulfacrylate), localized areas of cell adhesion were observed; however, a portion of the population also tended to form clusters. In Group 3 (Histoacryl), the cells retained their typical elongated morphology characteristic of stromal cells and were evenly distributed across the substrate, which was comparable to the control group (no adhesive application).

Cell Viability Assessment (Annexin V-FITC/PI).

Flow cytometry analysis of cell viability revealed statistically significant differences among the groups in terms of necrosis and apoptosis levels:

• Necrosis levels were highest in Group 1 (Hemocompat), reaching 16.65%, which was statistically significantly higher than in the other groups ($p < 0.001$). In Group 2 (Sul-facrylate) and Group 3 (Histoacryl), the necrosis levels were 1.56% and 3.17%, respectively, while in the control group the value did not exceed 1.32%.

• Apoptosis in Group 1 was characterized by elevated levels of both early (10.45%) and late (17.4%) apoptosis, with a total apoptotic index of 27.85%. In Group 3 (Histoacryl), the highest level of early apoptosis was observed (44.48%), which may indicate cellular stress while maintaining recovery potential. Late apoptosis in this group was 3.75%, with a combined total of 48.23%. In Group 2, total apoptosis reached 28.67%. The control group showed minimal apoptosis values (1.41%), confirming the preservation of cellular homeostasis in the absence of adhesive materials.

Gating diagrams and quantitative bar charts are shown in Figures 4 and 5, confirming statistically significant differences between the groups ($p < 0.001$, ANOVA, Tukey's test).

Assessment of Proliferative Activity via Ki-67 Marker.

Cell proliferative activity was assessed using staining for the nuclear marker Ki-67 followed by flow cytometric analysis. In the control group, the proportion of Ki-67-positive cells was the highest and served as the reference value. In Group 1 (Hemocompat), a statistically significant reduction in Ki-67-positive cells was observed ($p < 0.001$), indicating suppressed proliferative activity, most likely due to the cytotoxic effect of the adhesive. In Groups 2 and 3 (Sul-facrylate and Histoacryl), a reduction in Ki-67 levels was also noted, although it did not reach statistical significance compared to the control. These findings are illustrated in Figures 6 and 7 and Table 2.

Thus, based on the obtained data, it can be concluded that the feracrylate-based adhesive exhibited the most pronounced cytotoxic effect, manifested in reduced proliferation, increased apoptosis and necrosis levels, and impaired cell morphology. In contrast, cyanoacrylate-based adhesives—especially the imported Histoacryl—demonstrated satisfactory biocompatibility under the conditions of this experiment.

Discussion.

The obtained results indicate a pronounced variability in the cytotoxic properties of the investigated adhesive compositions. Samples from Group 1 (Hemocompat, based on feracrylate) showed the most significant reduction in cell proliferative activity, as evidenced by both morphological changes and a decrease in Ki-67 marker expression. This suggests that feracrylate-based adhesive exerts a suppressive effect on the regenerative potential of stromal cells.

In contrast, cyanoacrylate-based adhesives (particularly Histoacryl) demonstrated the most bio-compatible behavior toward the cell culture, maintaining typical morphology and a high per-centage of Ki-67-positive cells. These findings support their potential suitability for use as wound dressings in dental surgical practice.

Comparative analysis of apoptosis and necrosis levels revealed statistically significant differences between the groups, particularly regarding late apoptosis and necrotic cell death. The highest levels of these processes were observed in response to the feracrylate-based adhesive, suggesting that it may be a less favorable material for use in sensitive areas of the oral mucosa.

In this study, two null hypotheses were tested. The first null hypothesis (H_{01})—that the tested adhesives have no effect on the proliferative activity of stromal cells—was rejected, as a statistically significant decrease in Ki-67 expression was observed in the feracrylate group ($p < 0.001$). The second hypothesis (H_{02})—that the levels of apoptosis and necrosis are equal across all groups—was also rejected due to the identification of statistically significant differences among groups (ANOVA, $p < 0.001$).

The obtained data align with the findings with the demonstration that n-butyl cyanoacrylate-based adhesives exhibit lower cytotoxicity compared to acrylic-based alternatives when in contact with gingival mesenchymal cells [9]. Similar conclusions were reported with improved biocompatibility of n-butyl cyanoacrylate in soft tissue applications, particularly in periodontal and mucogingival procedures [10].

A comparable trend was described with the conduction in vitro testing on osteoblast-like cells and found that ethyl-cyanoacrylate used within a therapeutic dosage did not impair cellular proliferation, although it induced transient early apoptosis without progression to necrosis [11]. These results are consistent with the findings observed in Group 3 of our study. Furthermore, prior work confirmed that short-term exposure to ethyl-cyanoacrylate activates controlled apoptotic pathways while maintaining overall cell viability in bone-derived cell cultures [12].

An important observation is that the high level of early apoptosis identified with the use of His-toacryl was not accompanied by an increase in the necrotic cell population. This may indicate a cellular stress response followed by recovery in the absence of aggressive cytotoxic damage. A similar mechanism was described by Choi et al. [13], who assessed cellular responses to aldehyde-based surgical adhesives. Moreover, Miki et al. [14] reported comparable findings, suggesting that transient

Table 2. *Summary of Statistical Results of Proliferation (Ki-67), Apoptosis, and Necrosis Assessment. p-values were determined by ANOVA followed by Tukey's post hoc test. Different super-script letters indicate statistically significant differences (p<0.05) between groups.*

Experimental Group	Ki-67-positive cells (%)	Necrosis (%)	Early Apoptosis (%)	Late Apoptosis (%)	Total Apoptosis (%)
Control	82.4 ± 3.1^a	1.32 ± 0.15^a	0.92 ± 0.11^a	0.49 ± 0.08^a	1.41 ± 0.19^a
Hemocompat	45.7 ± 4.5^b	16.65 ± 1.23^b	10.45 ± 0.89^b	17.40 ± 1.21^b	27.85 ± 2.10^b
Sulfacrylate	71.2 ± 2.7^a	1.56 ± 0.17^a	16.12 ± 1.05^c	12.55 ± 0.77^c	28.67 ± 1.35^b,c
Histoacryl	74.9 ± 3.2^a	3.17 ± 0.22^a	44.48 ± 2.12^d	3.75 ± 0.41^a	48.23 ± 2.05^d

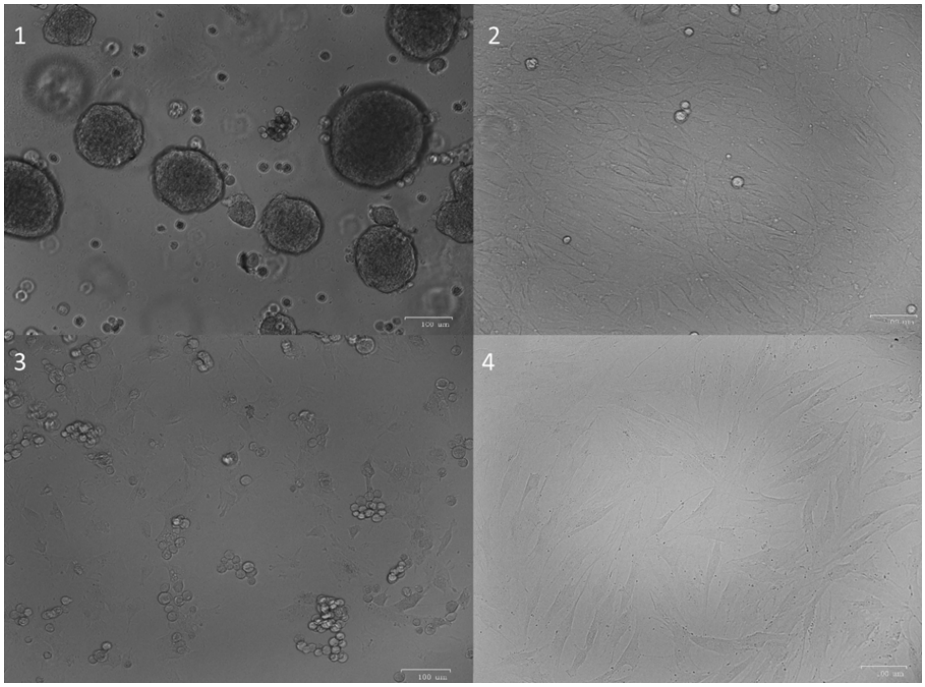


Figure 3. Microscopy of cells after 24-hour incubation with adhesive samples. 1 – Hemocompat, 2 – Histoacryl, 3 – Sulfacrylate, 4 – Control.

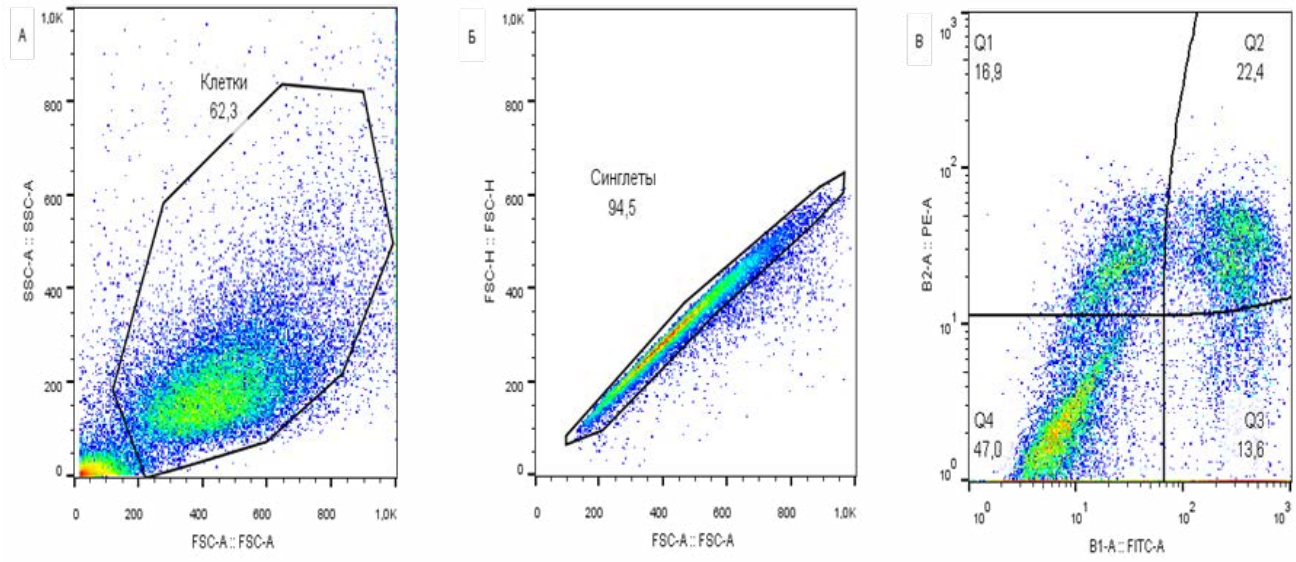


Figure 4. *Flow cytometry gating strategy for apoptosis and necrosis assessment.*

Representative gating strategy using Hemocompat-treated cells: (A) total cell population gate; (B) gating of single-cell events; (C) apoptosis and necrosis analysis identifying populations: Q1 – necrotic cells (Annexin V$^-$/PI$^+$), Q2 – late apoptotic cells (Annexin V$^+$/PI$^+$), Q3 – early apoptotic cells (Annexin V$^+$/PI$^-$), and Q4 – viable cells (Annexin V$^-$/PI$^-$)."

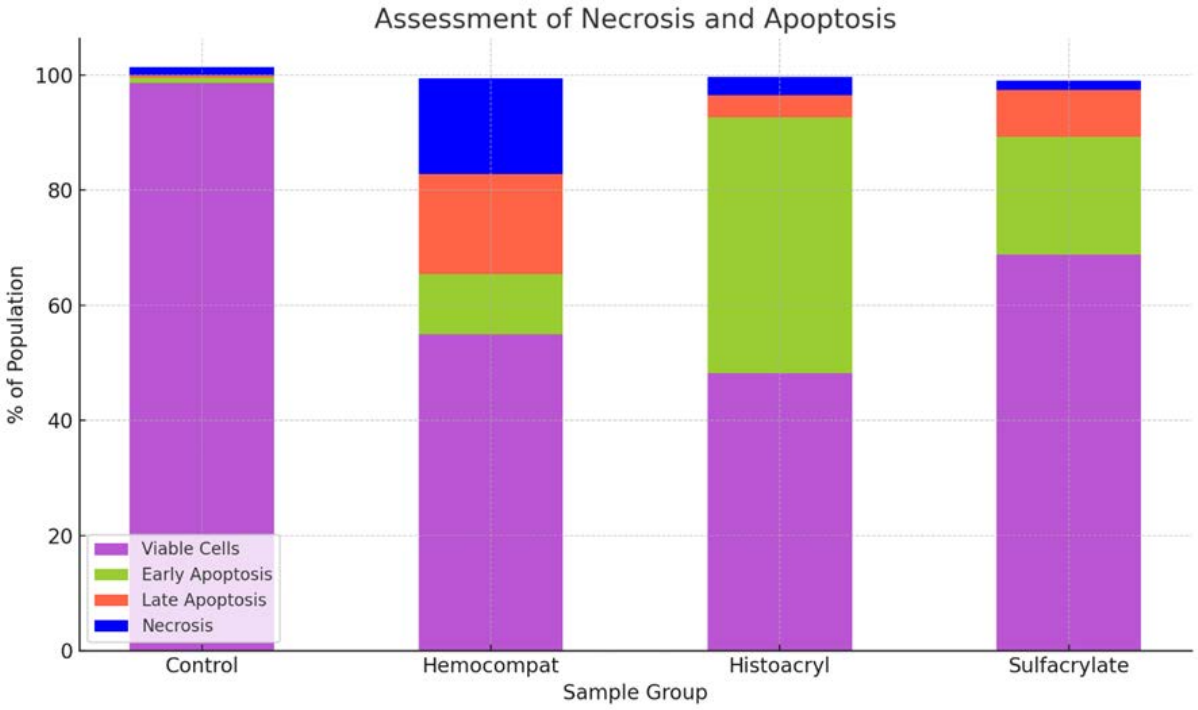


Figure 5. *Assessment of necrosis and apoptosis in the tested samples.*

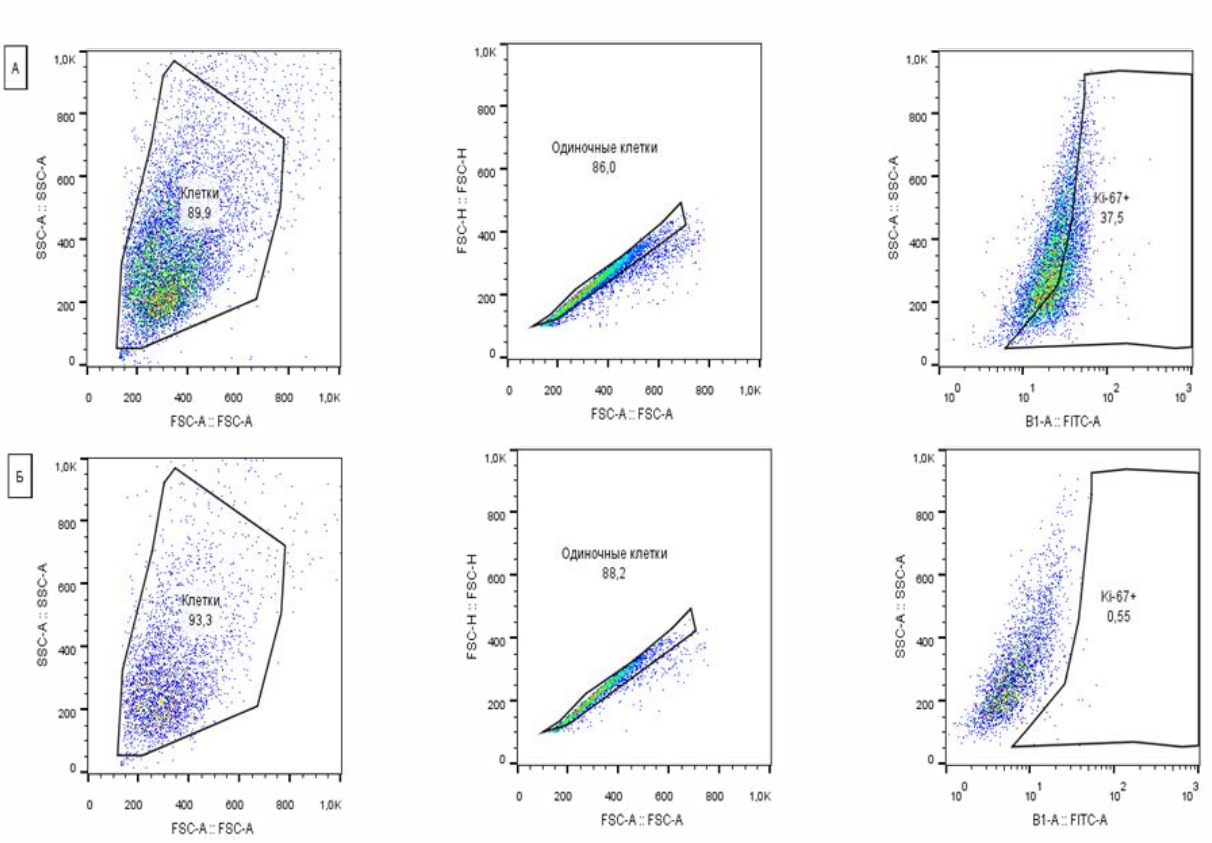
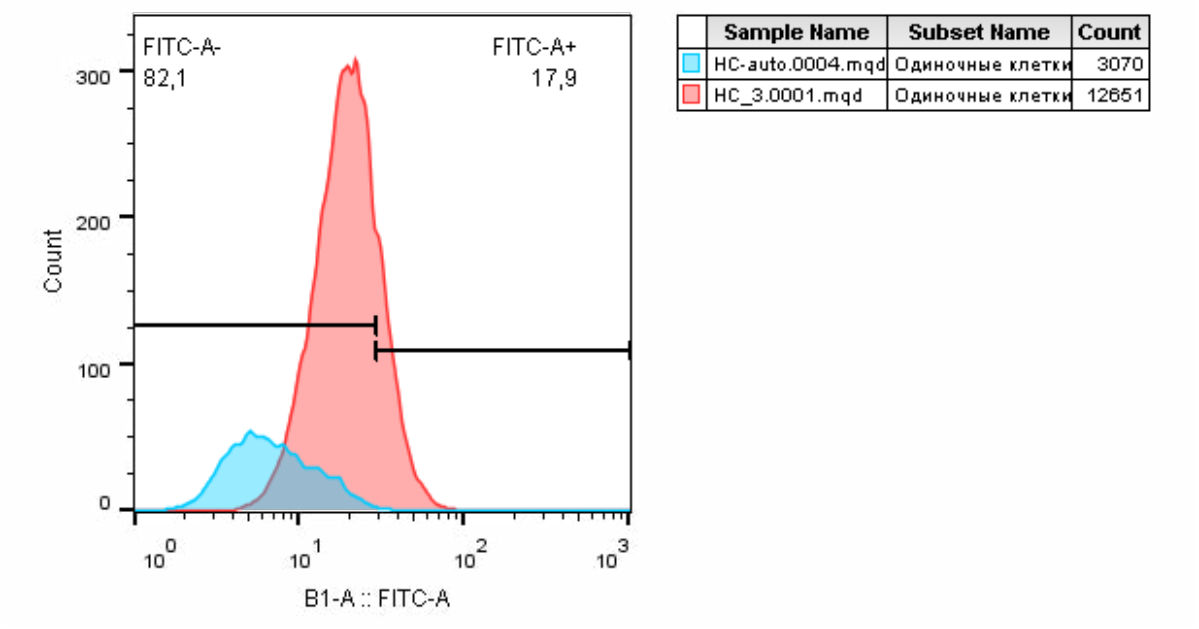


Figure 6. *Flow cytometry gating strategy for Ki-67 expression. Representative histograms showing Ki-67-positive cells across all groups: (A) Control; (B) Hemocompat; (C) Sulfacrylate; (D) Histoacryl. The percentages indicate the proportion of actively proliferating cells.".*



	Sample Name	Subset Name	Count
	HC-auto.0004.mqd	Одиночные клетки	3070
	HC_3.0001.mqd	Одиночные клетки	12651

Figure 7. *Ki-67 marker staining histogram for Group 1 sample.*

apoptotic signalling without necrosis may represent an adaptive response to moderate chemical stress in mucosal tissues.

On the other hand, the data obtained for the Hemocompat adhesive are consistent with the findings of Li et al. [15], who demonstrated that feracrylate-containing materials induce both apoptotic and necrotic death of oral epithelial cells. This effect was attributed to the generation of free radicals and disruption of mitochondrial homeostasis. Similar observations were made by Kim et al. [16], who identified oxidative stress as a key mediator of cytotoxicity in oral epithelial cells exposed to polymer-based hemostatic agents.

The morphological data obtained through microscopy confirm the quantitative findings of flow cytometry [25,26]: cells exposed to a feracrylate-containing medium lose their ability to ad-here. In vivo, this may lead to impaired epithelialization and delayed healing of the donor site. Similar inhibitory effects on cell adhesion and migration have been reported for feracrylate-based materials in several studies, including those by Park et al. [17] and Morita et al. [18], emphasizing their potential to interfere with tissue regeneration.

Particular attention should be given to the clinical application of cyanoacrylate adhesives in surgical dentistry. Studies by Kazancioglu et al. and Pogrel et al. [19] confirm that the use of n-butyl cyanoacrylate-based adhesives reduces healing time, alleviates pain, and does not exert a suppressive effect on local tissue proliferation. Furthermore, a randomized clinical trial by Cavalcante et al. [20] demonstrated favorable outcomes in terms of wound closure and patient comfort when cyanoacrylate adhesives were used following periodontal surgery.

However, despite the positive data obtained for cyanoacrylate-based adhesives, it is important to consider the dose-dependent nature of their effects. An increase in concentration, volume, or repeated application may trigger cellular stress responses. This underscores the importance of adhering to application protocols and the necessity for standardization of adhesive volume in clinical settings [21,22].

Thus, the obtained results support the conclusion of high biocompatibility of cyanoacrylate adhesives—particularly the imported product Histoacryl—and the limited applicability of feracrylate-containing compositions in the context of open oral mucosal wounds. The rejection of both null hypotheses confirms the significant influence of adhesive materials on cellular physiology, which must be carefully considered when selecting products for surgical dental procedures [23,24].

Conclusion.

The conducted study demonstrated significant differences in the biocompatibility of medical adhesives used in surgical dentistry when interacting with a culture of human oral mucosal stromal cells. The feracrylate-based adhesive (Hemocompat) exhibited the most pronounced cytotoxic effects, including a statistically significant reduction in cellular proliferative activity (as indicated by Ki-67 levels), increased levels of both early and late apoptosis, and a high level of necrosis. These findings suggest limited clinical applicability of this material, particularly in procedures involving donor sites of the oral mucosa.

Cyanoacrylate-based adhesives (Sulfacrylate and Histoacryl) demonstrated significantly higher levels of biocompatibility. In particular, the imported product Histoacryl provided the most favorable results in terms of cell morphology, minimal signs of necrosis, and stable proliferative activity comparable to the control group. This supports its consideration as a preferred material for wound coverage in oral mucosal applications.

Both null hypotheses proposed in this study were rejected. Statistically significant differences were observed among the tested adhesives in terms of proliferation (Ki-67), as well as apoptosis and necrosis levels, indicating varying degrees of impact on cellular physiology.

Clinical Relevance and Study Limitations.

The clinical significance of this study lies in its potential to support a more informed selection of adhesive materials during soft tissue procedures in the oral cavity, particularly in the

harvesting of free gingival grafts. Biocompatibility and minimal cytotoxicity of the adhesive are directly associated with the speed and quality of tissue regeneration.

Study limitations include:

• The exclusive use of an *in vitro* model, which does not account for the complex micro-environment of inflammation, microbiota, and mechanical forces present in the oral cavity.

• The use of only one cell type (stromal cells), without evaluating the effects on epithelial cells, fibroblasts, or immune cells.

• A limited sample size and number of tested adhesive compositions (three materials).

• A short observation period—analysis was conducted within 24–48 hours, without monitoring delayed or long-term effects.

In the future, extended *in vivo* studies are planned using animal models and/or clinical cases, along with the analysis of additional biomarkers of cellular activity and tissue healing.

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АНАЛИЗ БИОМАРКЕРА ПРОЛИФЕРАЦИИ КУЛЬТУРЫ СТРОМАЛЬНЫХ КЛЕТОК ПРИ ИСПОЛЬЗОВАНИИ МЕДИЦИНСКИХ КЛЕЕВ

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Аннотация

Введение. Медицинские клеи активно применяются в хирургической стоматологии, в том числе для покрытия донорских зон после забора десневых трансплантатов. Однако их цитотоксические свойства и влияние на пролиферативную активность тканей остаются предметом научных обсуждений.

Цель. Оценить влияние различных клеевых композиций на пролиферативную активность стромальных клеток слизистой оболочки полости рта человека методом окрашивания на Ki-67, а также проанализировать апоптотические и некротические изменения.

Материалы и методы. В исследование включены три типа **медицинских клеев:** феракрил (Гемокомпакт), отечественный цианакрилат (Сульфакрилат) и импортный цианакрилат (Гистоакрил). Клеи наносили на стерильные поликарбоксилатные пластины, к которым подсевали стромальные клетки человека. Через 24–48 часов инкубации проводили морфологическую оценку, анализ апоптоза и некроза с использованием Annexin V-FITC/PI и определение экспрессии Ki-67 методом проточной цитометрии. Статистическая обработка включала ANOVA и пост-хок тест Тьюки ($p<0,05$).

Результаты. Образцы с феракрилом показали наибольшую цитотоксичность: высокий уровень некроза (16,65%) и снижение Ki-67 ($p<0,001$). Цианакрилатные клеи обеспечивали более благоприятный профиль биосовместимости: Гистоакрил демонстрировал минимальный уровень некроза и наибольшую сохранность морфологии клеток. Обе нулевые гипотезы были отклонены.

Вывод. Клеи на основе цианакрилатов, особенно импортного производства, являются более безопасными для использования в зонах повышенной регенеративной активности. Клей на основе феракрила ограничен в применении из-за высокого цитотоксического действия. Полученные данные обосновывают выбор клеевых материалов при мягкотканевых вмешательствах.

Ключевые слова: Ki-67, медицинские клеи, стромальные клетки, апоптоз, пролиферация, цитотоксичность, проточная цитометрия.

რეზიუმე

სამედიცინოწებოებითსტიმულირებულსტრომალური უჯრედების კულტურის პროლიფერაციული ბიომარკერის ანალიზი

ხაბაძე ზ. ს, ბაკაევი იუ.ა, მარდანოვი ო. ს, ლოხონინა ა. ვ, ივინა ა. ა, ბადალოვი ფ. ვ, უმაროვი ა. იუ, ვეჰბი აჰმად, კაკაბაძე ე. მ, დაშტიევა მ. იუ.

ანოტაცია

შესავალი. სამედიცინო წებოები ფართოდ გამოიყენება ქირურგიულ სტომატოლოგიაში, მათ შორის, ღრძილების ტრანსპლანტატის აღების შემდეგ დონორის ზონის დასაფარად. თუმცა, მათი ციტოტოქსიური თვისებები და გავლენა ქსოვილის პროლიფერაციულ აქტივობაზე კვლავ რჩება სამეცნიერო განხილვის საგნად.

მიზანი. სხვადასხვა წებოვანი კომპოზიციების გავლენის შეფასება ადამიანის პირის ღრუს ლორწოვანი გარსის სტრომალურ უჯრედებზე, Ki-67 მარკერის გამოყენებით, ასევე აპოპტოზური და ნეკროზული ცვლილებების ანალიზი.

მასალები და მეთოდები. გამოკვლევაში შედიოდა სამგვარიანი წებო: ფერაკრილის (ჰემოკომპაქტი), ადგილობრივი წარმოების ციანაკრილატი (სულფაკრილატი) და იმპორტული ციანაკრილატი (ჰისტოაკრილი). წებოები ინახებოდა სტერილურ პოლიკარბოქსილატურ ფირფიტებზე, რომლებზეც ითესებოდა ადამიანის სტრომალური უჯრედები. 24–48 საათიანი ინკუბაციის შემდეგ ჩატარდა მორფოლოგიური შეფასება, Annexin V-FITC/PI ტესტით აპოპტოზისა და ნეკროზის ანალიზი, ასევე Ki-67-ის ექსპრესიის განსაზღვრა პროტოქზიტომეტრიის მეთოდით. სტატისტიკური ანალიზი მოიცავდა ANOVA-ს და ტიუკის პოსტ-ჰოკ ტესტს ($p<0.05$).

შედეგები. ფერაკრილის ჯგუფმა აჩვენა უმაღლესი ციტოტოქსიკურობა: ნეკროზის მაღალი დონე (16.65%) და Ki-67-ის კლება ($p<0.001$). ციანაკრილატური წებოები გამოირჩეოდნენ უკეთესი ბიოშეთავსებადობით; ჰისტოაკრილმა აჩვენა მინიმალური ნეკროზი და უჯრედების მორფოლოგიის მაქსიმალური შენარჩუნება. ორივე ნულოვანი ჰიპოთეზა უარყოფილი იქნა.

დასკვნა. ციანაკრილატის საფუძველზე შექმნილი წებოები, განსაკუთრებით იმპორტული წარმოების, უფრო უსაფრთხოა მაღალი რეგენერაციული აქტივობის ზონებში გამოსაყენებლად. ფერაკრილის საფუძველზე დამზადებული წებოები შეზღუდულია კლინიკურ გამოყენებაში მათი მაღალი ციტოტოქსიკურობის გამო. მიღებული მონაცემები ხელს უწყობს წებოვანი მასალების სწორად შერჩევას რბილი ქსოვილის ქირურგიულ ჩარევებში.

საკვანძო სიტყვები: Ki-67, სამედიცინო წებოები, სტრომალური უჯრედები, აპოპტოზი, პროლიფერაცია, ციტოტოქსიკურობა, პროტოქზიტომეტრია.